Caveats on the analysis of indigoid dyes by mass spectrometry

P E McGovern, J Lazar* and R H Michel

Museum Applied Science Center for Archaeology, University of Pennsylvania, Philadelphia, PA 19104, USA

** E I DuPont de Nemours & Co., Wilmington, DE 19898, USA*

We recently reported in this journal [1] that indigoid dyes can be analysed by mass spectrometry either directly from the fibre by volatilisation of the dye or indirectly from the residue of a solvent extract. We also stated that an important advantage of these methods in the analysis of haloindigoids is that they are not complicated by photodehalogenation, a side reaction that is quite likely to occur when a reductive (vat) extraction method is employed.

Recently, however, more extensive experimentation has shown that volatilisation of 6,6′-dibromoindigotin (DBI) from a fibre can result in considerable monodebromination, to form monobromoindigotin (MBI). Small amounts of the double debrominated product, indigotin, have also been found, although its presence is frequently obscured by numerous other lower molecular mass materials. Furthermore, contrary to the statement in our earlier article that pointed up the advantages of steam treating fibres before mass spectrometry, we are now able to detect DBI on unsteamed fibres by mass averaging the appropriate multiple scans, which were run as previously described [1].

In order to elucidate the debromination of DBI during mass spectrometric analysis in the presence of other organic compounds, several samples were examined with the dye covered by fibrous materials. The tests are summarised in Table 1 (samples 2–6). In contrast to the analysis of synthetic DBI alone (sample 1), nearly all of the covered samples show distinct bands for MBI. The presence of indigotin is less clear, because of contamination by lower molecular mass materials. Yet, as seen in Figure 1 (top), indigotin is definitely less prevalent than MBI. This is the expected result for the early stages of a sequential debromination process in which DBI is still strongly detected and both debromination steps are assumed to go at similar rates. Thus, unless DBI is analysed as the pure dye, the presence of other organics, whether in the fibre or in the extraction mixture, can conceivably give debromination products. On the other hand, if the indigotin content were found to be high compared with that of MBI, and the DBI content were high as well, it could be concluded that indigotin was present in the original sample and was not solely a debromination product.

Table 1 Mass spectra bands resulting from the analysis of DBI

	Strength of indigotin and MBI bands relative to that of DBI bands (*M/Z* 418, 420, 422)	
Sample	Indigotin (*M/Z* 262)	MBI (*M/Z* 340, 342)
1. Synthetic DBI (recovered from DMSO) (Figure 1, bottom)	None	None
2. Dry filter paper	None	Weak
3. Moist filter paper (Figure 1, top)	Weak	Moderate
4. Flax fibre	Background interference (certainly weak)	Moderate
5. Wool fibre	Background interference (certainly weak)	Moderate
6. Silk fibre	Background interference (certainly weak)	Moderate
7. Mexican cotton fibre dyed with *Purpura patula pansa* secretions (Figure 2, top)	Background interference (certainly weak)	Strong
8. Same Mexican fibre (DMSO extracted) (Figure 2, bottom)	Moderate	Weak

Samples 2–8: synthetic DBI covered with the following packing material, and analysed by direct volatilisation:

The bromoindigoids apparently react in some way with the cellulosic or proteinaceous materials present in the fibres as the temperature of the mass spectrometer tube is progressively raised to about 450°C and the dyes are volatilised. Because DBI in the absence of such materials yields only DBI by this analysis (Figure 1, bottom; Table 1, sample 1), the substrate must enter into the

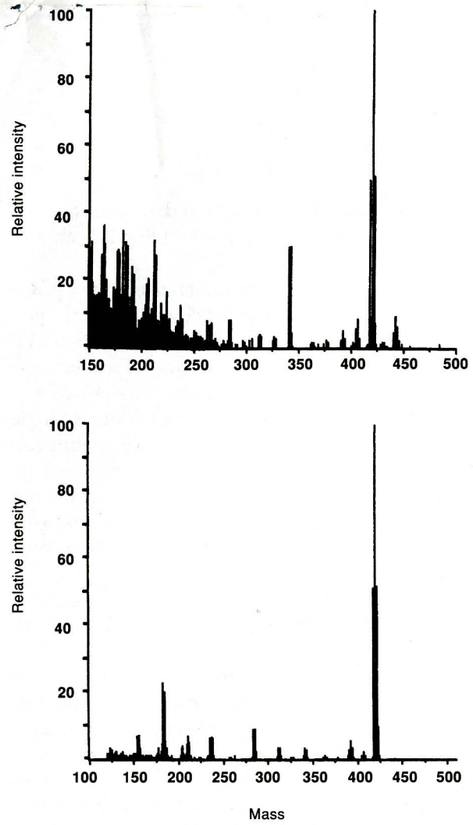

Figure 1 Synthetic DBI analysed with moist filter paper packing mass spectrometer sample tube (top), and after recovery from dimethylsulphoxide solution (bottom)

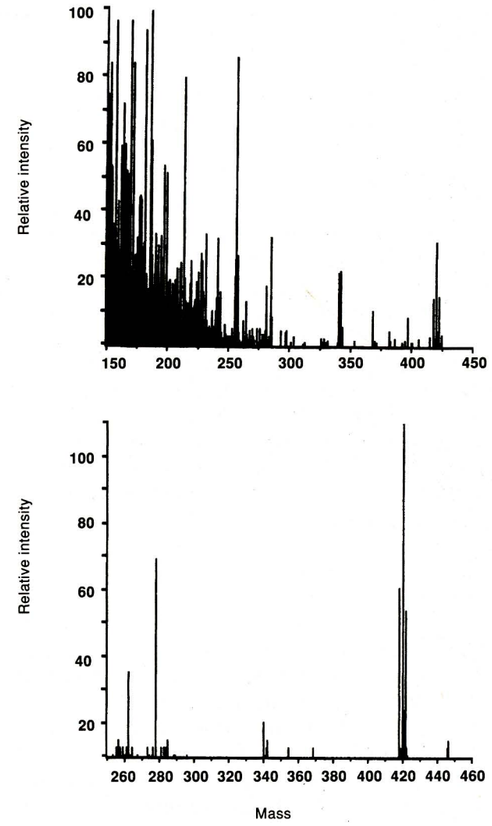

Figure 2 Mexican cotton fibre dyed with DBI from *Purpura patula pansa* analysed by direct volatilisation (top), and from dimethylsulphoxide extract (bottom)

reaction(s). A physical factor, which may involve the residence time of the indigoid in the vicinity of the substrate at high temperatures, is probably also important in the process, since tubes with more tightly packed fibres exhibit more dehalogenation (cf. samples 2 and 3 in Table 1).

Figure 2 (top) gives an analysis by direct volatilisation (Table 1, sample 7) of a Mexican cotton fibre dyed with DBI from the Pacific Ocean mollusc *Purpura patula pansa*. The strengths of the DBI and MBI bands are similar to each another, while the indigotin band is so weak that it is difficult to distinguish from the background. Figure 2 (bottom) shows the spectrum of the dimethylsulphoxide (DMSO) extraction of the same Mexican fibre. The indigotin band can be seen to exceed a questionable MBI band, the opposite result of what would be expected if only sequential debromination of DBI were involved. The implication of this finding is that *Purpura patula pansa* secretions already contain small amounts of indigotin; only one other related mollusc, the Old World species *Murex trunculus*, has been reported to yield a mixture of DBI and indigotin [2].

In our most recent analytical work on the natural products of secretions from the *M trunculus* and *M brandaris*, as yet unpublished, we have preferred DMSO to quinoline as an extractant, because of DMSO's greater solvent power and volatility at lower temperatures. In our ongoing investigation of indigoid dye processing in antiquity [3], the refinement of analytical techniques plays a crucial role in establishing the composition of the starting materials and subsequent physical and chemical changes of the dyestuffs.

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